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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
09/621,448	07/21/00	O'DONOHUE M	1533.1010002

HM22/0405

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EXAMINER	
STEADMAN, D	
ART UNIT	**PAPER NUMBER**
1652	9

DATE MAILED: 04/05/01

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No.	Applicant(s)	
	09/621,448	O'DONOHUE ET AL.	
	Examiner	Art Unit	
	David J. Steadman	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____ .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-23* is/are pending in the application.

4a) Of the above claim(s) *14-17* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13 and 18-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claims *1-23* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

Attachment(s)

15)☒ Notice of References Cited (PTO-892)
16)☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
17)☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
18)☐ Interview Summary (PTO-413) Paper No(s). _____ .
19)☐ Notice of Informal Patent Application (PTO-152)
20)☐ Other:

DETAILED ACTION

Election/Restrictions

Restriction to one of the following inventions is required under 35 U.S.C. 121:

I. Claims 1-13 and 18-23, drawn to a method of producing L-amino acids using an altered bacterial cell, classified in class 435, subclass 115.

II. Claims 14 and 17, drawn to a vector and host cell expressing a mutant phosphoglucose isomerase polypeptide, classified in class 435, subclass 252.3.

III. Claims 15 and 16, drawn to a method of producing a bacterial cell with a mutated phosphoglucose isomerase (*pgi*) gene, classified in class 435, subclass 477.

The inventions are distinct, each from the other because:

Groups I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (MPEP § 806.05(h)). In the instant case the vector of Group II can be used for protein expression.

The methods of Groups I and III are independent as they comprise different steps, utilize different products and yield different results.

Groups II and III are related as process of making and product made. The inventions are distinct if either or both of the following can be shown: (1) that the process as claimed can be used to make other and materially different product or (2) that the product as claimed can be made by another and materially different process (MPEP § 806.05(f)). In the instant case the

bacterial cell with a mutant phosphoglucose isomerase gene can be made by chemical mutagenesis.

Because these inventions are distinct for the reasons given above, have acquired a separate status in the art and **require a separate search** resulting in an undue search burden on the Examiner, restriction for examination purposes as indicated is proper.

Applicant is advised that the reply to this requirement to be complete must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a petition under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

During a telephone conversation with Steven Ludwig on 02/05/01 a provisional election was made **without** traverse to prosecute the invention of Group I, claims 1-13 and 18-23. Affirmation of this election must be made by applicant in replying to this Office action. Claims 14-17 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Drawings

1. The drawings submitted with this application have not been reviewed by a draftsperson at this time. When formal drawings are submitted, the draftsperson will perform a review. Direct any inquiries concerning drawing review to the Drawing Review Branch (703) 305-8404.

Claim Objections

2. Claims 7, 8, 12, 20, and 21 are objected to because of the recitation of "pgi". Abbreviations, unless otherwise obvious and/or commonly used in the art, should not be recited in the claims without at least once reciting the entire phrase, i.e., "phosphoglucose isomerase" for which the abbreviation is used. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 2-5, 9, 10, and 12 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

4. The terms "increased carbon flux" in claim 2, "increased amount" in claims 3, 9, and 10, "decreased carbon flux" in claim 4, and "decreased amount" in claim 5 are unclear absent a statement defining to what the levels of carbon flux or enzymes are being compared. The terms are relative terms and the claims should define and clearly state as to what the carbon flux and enzyme levels are being compared (e.g., the altered cell has increased carbon flux through the oxidative branch of the pentose phosphate pathway compared to what?).

5. The term "a gene selected from the group consisting of a mutated pgi gene" in claim 12 is unclear and confusing as a group does not have only a single member. It is suggested that the language "a gene selected from the group consisting of a mutated pgi gene" be replaced with, for example, "a mutated pgi gene".

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims 1-13 and 18-23 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 1 (claims 3, 5, 6, and 9-11, and 13 dependent thereon), 2, 4, 7, 8, 12, 15, 18 (claims 19, 22, and 23 dependent thereon), 20, and 21 are directed to methods of producing L-amino acids by culturing an altered bacterial cell having an increased amount of NADPH (claim 1), an altered bacterial cell having increased flux through the oxidative branch of the pentose phosphate pathway (claim 2), an altered bacterial cell having decreased flux through the glycolytic pathway (claim 4), an altered bacterial cell having a decreased amount of 6-phosphoglucose isomerase (claims 5 and 18), an altered bacterial cell having a mutant *pgi* gene (claims 7, 12 and 20), or an altered bacterial cell produced by subcloning an internal region of a *pgi* gene and inserting said resulting vector into a bacterial genome via homologous recombination (claims 8 and 21). The specification teaches only a single representative species

of an altered bacterial cell having an increased amount of NADPH, an altered bacterial cell having increased flux through the oxidative branch of the pentose phosphate pathway, an altered bacterial cell having decreased flux through the glycolytic pathway, an altered bacterial cell having a decreased amount of 6-phosphoglucose isomerase, an altered bacterial cell having a mutant *pgi* gene, or an altered bacterial cell produced by subcloning an internal region of a *pgi* gene and inserting said resulting vector into a bacterial genome via homologous recombination, i.e., a *Coryneform glutamicum* cell with a disrupted *pgi* gene. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of an altered bacterial cell having an increased amount of NADPH, an increased flux through the oxidative branch of the pentose phosphate pathway, a decreased flux through the glycolytic pathway, a decreased amount of 6-phosphoglucose isomerase, a mutant *pgi* gene compared to an unaltered cell, or an altered bacterial cell produced by subcloning an internal region of a *pgi* gene and inserting said resulting vector into a bacterial genome via homologous recombination compared to an unaltered cell. Given this lack of description of representative species encompassed by the genus of the claims, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

7. Claims 1-7, 9-13, and 18-20 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of producing L-amino acids comprising: culturing an altered *Coryneform glutamicum* cell having a disrupted *pgi* gene, wherein L-amino acid yields from the altered cell are greater than yields from an unaltered cell does not reasonably provide enablement for a method of producing L-amino acids comprising: culturing an altered

bacterial cell with any *pgi* gene mutation or other alterations producing an increased level of NADPH compared to an unaltered cell, wherein L-amino acid yields from the altered bacterial cell are greater than yields from an unaltered cell. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

Claims 1-7, 9-13, and 18-20 are so broad as to encompass a method of producing L-amino acids comprising: culturing an altered bacterial cell having any alteration which produces increased levels of NADPH, including any mutated pgi gene. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of altered bacterial cells broadly encompassed by the claims. However, in this case, the disclosure is limited to a method of producing L-amino acids comprising: culturing an altered *Coryneform glutamicum* cell having a disrupted *pgi* gene.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompass a method of producing L-amino acids comprising: culturing an altered bacterial cell having any alteration which produces increased levels of NADPH because the specification does not

establish: (A) a rational and predictable scheme for obtaining increased L-amino acid yields from any altered bacterial cell having an increased amount of NADPH, as not all altered bacteria with increased levels of NADPH will have greater yields of L-amino acids; (B) regions of the *pgi* gene structure which may be modified with an expectation of obtaining a 6-phosphoglucose isomerase with the desired activity; (C) the general tolerance of 6-phosphoglucose isomerase to modification and extent of such tolerance; (D) a rational and predictable scheme for modifying any *pgi* gene with an expectation of obtaining the desired biological function; and (E) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a method of producing L-amino acids comprising: culturing an altered bacterial cell with any alteration which increases the level of NADPH including any mutated *pgi* gene, wherein L-amino acid yields from the altered cell are greater than yields from an unaltered cell. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 102/103

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

8. Claims 1, 2, 4-7, and 18-20 are rejected under 35 U.S.C. 102(b) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Mascarenhas et al. (Appl Environ Microbiol 57:2995-2999). Claims 1, 2, 4-7, and 18-20 are drawn to methods of producing L-amino acids by culturing an altered bacterial cell having an increased amount of NADPH (claim 1) or a decreased amount of 6-phosphoglucose isomerase relative to an unaltered cell (claim 18), wherein L-amino acid yields from the altered cell are greater relative to an unaltered cell, and optionally, wherein the altered cell has increased carbon flux through the oxidative branch of the pentose phophate pathway (claim 2), and optionally, wherein the bacterial cell has a decreased carbon flux through the glycolytic pathway (claim 4), and optionally, wherein the altered cell has a decreased amount of 6-phosphoglucose isomerase activity (claim 5), and optionally, L-amino acid yields from the altered cell are 1-100 % greater relative to the unaltered cell (claims 6 and 19) and optionally, wherein the altered cell has a mutant *pgi* gene (claims 7 and 20). Mascarenhas et al. teach a method of producing L-tryptophan using a genetically modified strain of *Escherichia coli* K12 W3110 (p 2995, Introduction, paragraph 4 and Materials and Methods, paragraph 2) with a deletion of the gene encoding phosphoglucose isomerase (p 2995, Abstract) by incubating the bacteria in a medium containing D-[6-^{13}C]glucose (p 2997, right column, paragraph 3). Mascarenhas et al. teach the conversion of glucose to tryptophan was twice as efficient in the bacteria with a deletion of *pgi* (p 2998, left column, paragraph 2 and p 2999,

Table 2) and the bacterial strain with a deletion of *pgi* increased the average rate of tryptophan production and accumulation of final product (p 2998, Results, final paragraph) by a 2:1 ratio (p 2998, left column, paragraph 2). This anticipates claims 18-20 as written.

Mascarenhas do not explicitly teach methods of producing L-amino acids by culturing an altered bacterial cell having an increased amount of NADPH relative to an unaltered cell, wherein the altered cell has increased carbon flux through the oxidative branch of the pentose phophate pathway, or decreased carbon flux through the glycolytic pathway. However, Mascarenhas et al. suggest that deleting the *pgi* gene alters the path of glucose catabolism by diverting carbon flow through the hexose monophosphate (HMP) shunt (p 2995, Abstract), i.e., pentose phosphate pathway. Mascarenhas et al. suggest the effect of deleting *pgi* is increased carbon flow through the HMP shunt resulting in the accelerated generation of NADPH, which serves to drive the biosynthesis of aromatic intermediates and glutamate (p 2998, Discussion, paragraph 3). Mascarenhas et al. suggest that using bacteria with a deleted *pgi* gene can have a dramatic effect on the biosynthetic capabilities of this bacterium (p 2998, Discussion, paragraph 1). In view of the disclosed suggestions by Mascarenhas et al., claims 1, 2, and 4-7 would have been obvious to one of ordinary skill in the art at the time of the invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. Claims 3, 9-13, 22, and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mascarenhas et al. in view of Voet et al. (Biochemistry 2nd Edition, Wiley and Sons, 1995, NY), Ishino et al. (IDS reference AR2; J Gen Appl Microbiol 37:157-165), and Marx et al. (IDS reference AT2; Biotech Bioeng 49:111-129). Claims 3, 9-13, 22 and 23 are drawn to methods for producing L-amino acids by culturing an altered bacterial cell having an increased amount of NADPH or a decreased amount of 6-phosphoglucose isomerase enzyme activity relative to an unaltered cell, wherein L-amino acid yields from the altered cell are greater relative to an unaltered cell, wherein the altered cell has an increased amount of one or more of glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase (claim 3), the altered cell has an increased amount of malic enzyme (claim 9), the altered cell has an increased amount of isocitrate dehydrogenase (claim 10), the altered cell is a *C. glutamicum* cell (claims 11 and 22), the *C. glutamicum* cell has a mutated *pgi* gene (claim 12), or the L-amino acid comprises L-lysine (claims 13 and 23).

Mascarenhas et al. disclose the teachings described above. Mascarenhas et al. do not teach a method of producing L-amino acids or lysine by culturing a *C. glutamicum* cell, a *C. glutamicum* cell with a mutated *pgi* gene, or an altered bacterial cell with: an increased amount of one or more of glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase, an increased amount of malic enzyme, or an increased amount of isocitrate dehydrogenase.

Voet et al. teach "NADPH is generated by the oxidation of [glucose-6-phosphate] via an alternative pathway to glycolysis, the pentose phosphate pathway (also called the hexose monophosphate (HMP) shunt" (p 617, right column, paragraph 2). Voet et al. additionally teach

"glycolysis is alternatively known as the Embden-Meyerhof-Parnas pathway" (p 445, left column, paragraph 3). Voet et al. further teach "The malic enzyme reaction resembles that of isocitrate dehydrogenase" (p 687, left column, paragraph 1) and that "Malic enzyme's conenzyme is NADP+, so that when this route is used NADPH is produced" (p 687, left column, paragraph 1).

Ishino et al. teach that *Coryneform glutamicum* has been used industrially to produce amino acids by fermentation processes (p 157, abstract) because *C. glutamicum* accumulates L-lysine in culture medium (p 157, abstract). Ishino et al. generally teach a comparison of carbon flux through the HMP and the Embden-Meyerhof-Parnas (EMP) pathways during glutamate and lysine production in *C. glutamicum.* Ishino et al. specifically teach that in *C. glutamicum,* carbon flux through the HMP pathway during lysine fermentation is significantly higher than carbon flux through the Embden-Meyerhof-Parnas (EMP) pathway (p 161, paragraph 3) and propose that increased carbon flux through the HMP pathway is due to an increased requirement for NADPH during lysine production (p 157, abstract). Ishino et al. further teach that the greater contribution of HMP, i.e., increased carbon flux through the HMP pathway, resulted in a greater yield of lysine (p 162, bottom). Ishino et al. teach that "three enzymes are known to generate NADPH in *C. glutamicum,* glucose-6-phosphate dehydrogenase in HMP, 6-phosphogluconate dehydrogenase in HMP and isocitrate dehydrogenase in the tricarboxylate (TCA) cycle" (p 163, paragraph 2).

Marx et al. teach glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase are enzymes that control carbon flux into the pentose phosphate pathway (p114, Fig 1 and p 115, Table I).

Based on the teachings of Ishino et al., Marx et al., and Voet et al., it would have been obvious to one of ordinary skill in the art at the time of the invention that, by over-expressing glucose-6-phosphate dehydrogenase, lactonase, 6-phosphogluconate dehydrogenase, isocitrate dehydrogenase, and/or malic enzyme, one could increase the product, i.e., NADPH of these enzymes in a bacterial cell. Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Mascarenhas et al., Voet et al., Ishino et al., and Marx et al. for a method of producing L-amino acids by culturing an altered bacterial cell with an increased amount of one or more of glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase, an increased amount of malic enzyme, or an increased amount of isocitrate dehydrogenase. One would have been motivated for a method of producing L-amino acids or lysine by culturing an altered bacterial cell with: an increased amount of one or more of glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase, an increased amount of malic enzyme, or an increased amount of isocitrate dehydrogenase because of the teachings of Mascarenhas et al., Voet et al., Ishino et al., and Marx et al. who collectively taught that by deleting the *pgi* gene, carbon flow through the HMP shunt is increased, resulting in the accelerated generation of NADPH, which serves to drive the biosynthesis of aromatic amino acid intermediates and glutamate and that the enzymes listed above are involved in the production of NADPH. One would have a reasonable expectation of success for a method of producing L-amino acids or lysine by culturing an altered bacterial cell with an increased amount of one or more of glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase, an increased amount of malic enzyme, or an increased amount of isocitrate dehydrogenase because of the results of Mascarenhas et al., and Ishino et al. Therefore, claims 3,

9, and 10, drawn to a method of producing L-amino acids or lysine by culturing an altered bacterial cell with an increased amount of one or more of glucose-6-phosphate dehydrogenase, lactonase, and 6-phosphogluconate dehydrogenase, an increased amount of malic enzyme, or an increased amount of isocitrate dehydrogenase would have been obvious to one of ordinary skill in the art.

Furthermore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Mascarenhas et al. and Ishino et al.for a method of producing L-amino acids or lysine by culturing a *C. glutamicum* cell or a *C. glutamicum* cell with a mutated *pgi* gene. One would have been motivated for a method of producing L-amino acids or lysine by culturing a *C. glutamicum* cell or a *C. glutamicum* cell with a mutated *pgi* gene because of the teachings of Mascarenhas et al. and Ishino et al. who taught *C. glutamicum* has been used industrially to produce amino acids by fermentation processes because *C. glutamicum* accumulates L-lysine in culture medium and that deleting the *pgi* gene results in increased carbon flow through the HMP shunt resulting in the accelerated generation of NADPH, which serves to drive the biosynthesis of aromatic intermediates and glutamate. One would have a reasonable expectation of success for a method of producing L-amino acids or lysine by culturing a *C. glutamicum* cell or a *C. glutamicum* cell with a mutated *pgi* gene because of the results of Mascarenhas et al. and Ishino et al. Therefore, claims 11-13, 22, and 23, drawn to a method of producing L-amino acids or lysine by culturing a *C. glutamicum* cell or a *C. glutamicum* cell with a mutated *pgi* gene would have been obvious to one of ordinary skill in the art.

10. Claims 8 and 21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mascarenhas et al. in view of Fitzpatrick et al. (IDS reference AS4; Appl Microbiol Biotechnol

42:575-580). Claims 8 and 21 are drawn to methods of producing L-amino acids by culturing an altered bacterial cell, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a bacterial genome via homologous recombination.

Mascarenhas et al. disclose the teachings described above. Mascarenhas et al. do not teach a method of producing L-amino acids by culturing an altered bacterial cell having an increased amount of NADPH, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a bacterial genome by homologous recombination.

Fitzpatrick et al. generally teach a method of inserting an internal fragment of a *C. glutamicum recA* gene into the genome of *C. glutamicum* by homologous recombination for the purpose of gene silencing. The method of Fitzpatrick et al. involves subcloning a fragment of the *recA* gene into a vector (p 578, under *Construction and characterization of recA mutants*) and integrating the *recA* gene fragment into the *C. glutamicum* genome by homologous recombination (p 578, under *Construction and characterization of recA mutants*).

Therefore, it would have been obvious to one of ordinary skill in the art to combine the teachings of Mascarenhas et al. and Fitzpatrick et al. for a method of producing L-amino acids by culturing an altered bacterial cell, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a bacterial genome via homologous recombination. One would have been motivated for a method of producing L-amino acids by culturing an altered bacterial cell, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a bacterial genome via homologous

recombination because of the teachings of Mascarenhas et al. and Fitzpatrick as described above. One would have a reasonable expectation of success for a method of producing L-amino acids by culturing an altered bacterial cell, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a bacterial genome via homologous recombination because of the results of Mascarenhas et al. and Fitzpatrick. Therefore, claims 8 and 21, drawn to a method of producing L-amino acids by culturing an altered bacterial cell, wherein the altered cell is produced by subcloning an internal region of a *pgi* gene and inserting the resulting vector into a bacterial genome via homologous recombination would have been obvious to one of ordinary skill in the art.

11. No claim is in condition for allowance.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David Steadman, whose telephone number is (703) 308-3934. The examiner can normally be reached Monday-Friday from 8:00 am to 4:30 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can be reached at (703) 308-3804. The FAX number for this Art Unit is (703) 308-4242. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Art Unit receptionist whose telephone number is (703) 308-0196.

David J. Steadman

REBECCA E. PROUTY
PRIMARY EXAMINER
GROUP ~~1800~~ 1600